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Effect of Repeated Applications of Nitrification Inhibitor on the Fraction and Group of Humus in Various Soils

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In a six-month incubation experiment, repeated application of KMP nitrification inhibitor to various soils decreased the percentage of fulvic acids in soil humus; in certain soils, it increased the percentage of humic acids and the humin content while broadening the C_{HA}/C_{FA} ratio. The inhibitor also decreased the percentage of highly condensed humic acids bound to potassium in the humus and increased the concentration of the less-condensed free humic acids while causing a decline in their degree of condensation.

Key words: Nitrification inhibitor, repeated applications, humus fractional composition, humus group composition

Nitrification inhibitors are now produced commercially in the United States, Germany, and Japan and are used extensively in many countries. In the Russian Federation, they are now used only to a minor degree. But they have been used regularly for rice growing in Krasnodar Kray.

There are few publications on the way in which systematic application of nitrification inhibitors affect soil humus. In small-plot experiments performed jointly by our institute and the All-Union Institute of Agricultural Microbiology, Sirota and Orlova [1] found that five annual applications of 2 kg/ha of KMP nitrification inhibitor to a sandy-loam Sod-Podzolic soil had no effect on total humus content, but did change the makeup of the humus, decreasing the percentage of fulvic acids and increasing the percentage of nonhydrolyzed residue. They therefore concluded that regular application of nitrification inhibitors decreased the mobility of soil organic matter. Kutuzova states [2] that the excess ammonium ion in the soil caused by nitrification inhibitors can be utilized in various ways by heterotrophic microorganisms, depending on the supply of readily

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Table 1

Some Agrochemical Characteristics of Soils before and after Incubation

Version*	Original					
	Humus, %	N_{tot}, %	Salt pH	Aqueous pH	$N\text{-}NH_4$, ppm	$N\text{-}NO_3$, ppm
1. Poorly cultivated Sod-Podzolic	1.50	0.088	5.2	6.4	11	7
2. The same with KMP	—	—	—	—	—	—
3. Well-cultivated Sod Podzolic	2.52	0.127	6.1	6.6	25	26
4. The same with KMP	—	—	—	—	—	—
5. Typical Chernozem	5.03	0.281	6.8	7.4	29	47
6. The same with KMP	—	—	—	—	—	—
7. Typical Sierozem	1.14	0.104	7.5	8.2	27	26
8. The same with KMP	—	—	—	—	—	—

Version*	After incubation				
	Humus, %	Aqueous pH	$N\text{-}NH_4$, ppm	$N\text{-}NO_3$, ppm	$N\text{-}NH_4$, % of $N\text{-}(NH_4 + NO_3)$
1. Poorly cultivated Sod-Podzolic	—	5.8	0	206	—
2. The same with KMP	1.57	7.6	178	0	100
3. Well-cultivated Sod Podzolic	—	5.3	0	315	—
4. The same with KMP	2.71	6.1	140	126	53
5. Typical Chernozem	—	7.2	0	219	—
6. The same with KMP	5.14	7.5	34.2	174	16
7. Typical Sierozem	—	8.2	0	188	—
8. The same with KMP	1.10	8.9	120	0	100

* The same numbering of versions is used in the remaining tables.

available organic matter. A deficiency of readily available carbon, combined with a high concentration of ammonia nitrogen, may increase the mobility of humic substances and their availability to the microflora, thus displacing the equilibrium between the synthesis and decomposition of soil organic matter toward the latter, while the presence of a sufficient supply of available carbon will

Table 2

Effect of Nitrification Inhibitor on Group Composition of Humus

Version	C_{tot}	Humic acids (HA)	Fulvic acids (FA)	Nonhydrolyzed residue	$C_{HA}:C_{FA}$	Humic acids	Fulvic acids	Nonhydrolyzed residue
	Percent by weight of soil					Percent of C_{tot}		
1	0,87	0,20	0,35	0,32	0,57	23	40	37
2	0,91	0,26	0,24	0,41	1,08	28	27	45
3	1,46	0,49	0,58	0,39	0,84	33	40	27
4	1,57	0,55	0,46	0,56	1,19	35	29	36
5	2,92	1,25	0,57	1,10	2,19	42	19	39
6	2,98	1,13	0,46	1,39	2,46	38	16	46
7	0,66	0,15	0,16	0,35	0,94	23	25	52
8	0,64	0,18	0,12	0,34	1,50	27	18	55

displace the equilibrium toward humus formation. Regular application of nitrification inhibitors to the soil increases their effect on heterotrophic microorganisms.

We investigated the effect of repeated application of nitrification inhibitor in humus quality in long term 6-month) incubation of various soils under optimum temperature and moisture conditions (28 °C, 60 percent of FMC). The samples were incubated in glass jars covered with perforated aluminum foil, each holding 200 g of dry soil; the experiment was performed in three replications. Before incubation, the soil in each jar was stirred with an aqueous solution of urea and KMP nitrification inhibitor at concentrations of 4 and 100 ppm (as nitrogen). After 1.5, 3, and 4.5 months, the same amount of KMP was added and mixed with the entire volume of soil. Thus, over the period of the experiment, the total application of KMP was 16 ppm. We studied heavy-loam Sod-Podzolic soil, heavy-loam Typical Chernozem, and long irrigated medium-loam Typical Sierozem; well-cultivated and poorly cultivated samples of the Sod-Podzolic soil were used. The specimens of Sod-Podzolic soils were collected from an unfertilized plot (for the poorly cultivated soil) and from a plot that had been fertilized annually for 55 years with high levels of mineral fertilizers and had been manured at two-year intervals; both plots were in long-term experimental fields of the DAOS. The Chernozem was collected from plots that had received no fertilizer in a long-term experimental field at the Grakov experimental area (Kharkov Oblast), and the Sierozem was collected from unfertilized control plots in an experimental area of the Samarkand Agricultural Institute.

After the soils were incubated, we measured the aqueous pH and determined the concentrations of ammonia nitrogen and nitrate nitrogen by the Bremner-Keeney steam distillation method [3], the fractional and group composition of the humus by a modification of the Tyurin method [4], and the optical densities of the humic acid fractions $\left(E_{C_{HA}}^{mg/ml}\right)$ by the Plotnikova-Ponomareva method [5]; a Specol spectrocolorimeter tuned to 465 nm and equipped with cuvettes with a thick-

Table 3

Effect of Nitrification Inhibitor on Fractional Composition of Humus

Version	Humic acids			Fulvic acids			Humic acids			Fulvic acids		
	I	II	III	I	II	III	I	II	III	I	II	III
	Percentage of soil by weight						Percentage of C_{tot}					
1	0,13	0,04	0,03	0,09	0,24	0,02	15*	5*	3	10**	28*	2**
2	0,21	0,02	0,03	0,05	0,18	0,01	23*	2*	3	6*	20*	1**
3	0,32	0,11	0,06	0,14	0,42	0,02	22**	7*	4	10*	29*	1
4	0,45	0,03	0,07	0,05	0,39	0,02	29*	2*	4	3*	25*	1
5	0,04	1,00	0,21	0,13	0,44	0,00	1**	34***	7	4	15*	0
6	0,05	0,91	0,17	0,11	0,35	0,00	2**	30**	6	4	12*	0
7	0,00	0,11	0,04	0,04	0,11	0,01	0	17*	6	6*	17*	2*
8	0,06	0,08	0,04	0,01	0,09	0,02	9	12*	6	1*	14*	3**

Note. Asterisks indicate significant difference between versions with and without inhibitor: *, at 0.05 level; **, at 0.10 level; ***, at 0.20 level.

ness of 10 nm was used. The data on the chemical composition of soils (in threefold replication) were analyzed by the variance method and the least significant difference (LSD) was calculated for each pair of versions (soil with inhibitor and control without inhibitor) of each soil.

After a half-year's incubation, all of the soils not treated with nitrification inhibitor were entirely devoid of exchange ammonium, and the nitrate nitrogen concentration was approximately equal to the amount of nitrogen that had been applied, except in the well-cultivated Sod-Podzolic soil (Table 1). Exchange ammonium was present only in the soils treated with the inhibitor. The smallest amount of nitrogen, representing 16 percent of total mineral nitrogen, was found in the well humified, biologically active Chernozem. In the well-cultivated Sod-Podzolic soil, the ammonia form comprised about half (53 percent) of the mineral nitrogen. In the poorly cultivated Sod-Podzolic soil and the Sierozem, all of the mineral nitrogen occurred as ammonia.

The concentration of mineral nitrogen was lower in the soils that had been treated with nitrification inhibitor and especially in the Sierozem. The apparent cause was increased immobilization of mineral nitrogen by the soil in the presence of the inhibitor. The increased biological incorporation of fertilizer nitrogen into soil organic matter is a typical and well-known property of the transformation of nitrogen in soil when nitrification inhibitors are prèsent [6].

The ammonia that built up in the soil in the presence of the inhibitor caused it to become more alkaline. The rise in pH was proportional to the amount of ammonia present. The greatest increase in pH (1.8 units) occurred in the poorly cultivated Sod-Podzolic soil, which after six months' incubation with the inhibitor contained 178 mg of ammonia nitrogen per kg. In the well-

cultivated Sod-Podzolic soil, which contained 140 ppm of ammonia nitrogen, the pH rose by 0.8 units, and in the Sierozem, containing 120 ppm of ammonia nitrogen, it rose by 0.7 units. The smallest rise in pH (0.3 units) occurred in the Chernozem, which after incubation had the lowest ammonia nitrogen content (34.2 ppm).

The fractional and group composition of the humus was determined by extracting specific humic acids (the sum of humic and fulvic acids) with 0.1 N NaOH solution, followed by their precipitation with 1.0 N H_2SO_4 and isolation of the following fractions: (I) the fraction directly extractable by 0.1 N NaOH, which contained the less-condensed free forms of the humic and fulvic acids and the forms bound to mobile sesquioxides; (II) the fraction containing more highly condensed humic acids, dissolved by 0.1 N NaOH only after decalcification of the soil (removal of exchange calcium), and polymer complexes of humic and fulvic acids, bound primarily to exchange calcium; (III) the humic acid fraction bound to clay minerals and to stable sesquioxides, which is extracted on heating with 0.02 N NaOH solution in a water bath.

The way in which six months' incubation of the soils with nitrification inhibitor affected the concentration of humus (C_{tot}) and its group composition is shown in Table 2. The inhibitor failed to produce a statistically significant effect on the concentration of C_{tot} in the poorly cultivated Sod-Podzolic soil, the Chernozem and the Serozem. A perceptible change in C_{tot} in the presence of the inhibitor was demonstrated only in the case of the well-cultivated Sod-Podzolic soil, but the change was significant only at the 0.02 level and the increase was only 0.1 percent (from 1.46 to 1.57 percent).

Treatment of the inhibitor produced an ambiguous change in the qualitative makeup of the humus in all soils, with a decline in the fulvic acid fraction, and increase in the nonhydrolyzed residue (humin), and an increase in the C_{HA}:C_{FA} ratio. In the Sod-Podzolic soils and the Sierozem, this increase in the ratio resulted not only from a significant decrease in the fulvic acid content of the humus, but also from a slight increase in its humic acid content.

Thus the results of our laboratory experiment are consistent with the field data of Sirota and Orlova [1], which indicate that the systematic application of nitrification inhibitor decreases the fulvate content of the humus and increases its humin content. In combination, these results suggest that changes in the qualitative makeup of humus as a result of regular application of nitrification inhibitor occur in all soil types.

A more complete indication of the effect of nitrification inhibitor on humus quality is given by the data on the fractional makeup of the humus (Table 3). In all the soils, the inhibitor decreased the absolute amount of fulvic acid fractions I and II in the soil while leaving fraction III essentially unaffected. It also increased the absolute amount of humic acid fraction I and decreased the amount of humic acid fraction II. Because in most soils the inhibitor did not affect the total humus content, the change in fractional makeup appears to have been primarily the result of redistribution among the fractions. Redistribution of humic acid fractions I and II of the Sod-Podzolic soil as a result of liming and cultivation is reported by Plotnikova and Orlova [7]. In our experiments, the increase in the concentration of humic acid fraction I in the presence of inhibitor apparently resulted from a decrease in the concentration of humic acid fraction II owing to a rise in the solubility of this fraction, which is the most valuable of the fractions.

Table 4

Effect of Nitrification Inhibitor on Optical Densities of Humic Acid Fractions

Version	Humic acid fraction		
	I	II	III
1	3,0	9,5	4,3
2	1,9	10,0	4,8
3	2,4	8,2	3,9
4	1,5	8,2	4,3
5	2,6	23,1	11,9
6	1,8	22,3	12,2
7	–	–	4,1
8	–	–	4,3

In addition to an increase in the amount of humic acid fraction I, the nitrification inhibitor also produced changes in the character of the fractions. The data in Table 4 show that the inhibitor decreased the optical density of humic acid fraction I by a factor of 1.4 in the Chernozem and by a factor of 1.6 in the Sod-Podzolic soils (humic acid fraction I was not present in the Sierozem). These data show that a decrease in the degree of condensation of humic acid fraction I was caused by the inhibitor; the optical density of humic acid fractions II and III was essentially unchanged.

Thus repeated application of nitrification inhibitor produces different effects on humus quality from soil to soil. In all soils, it decreases the mobility of the humus by decreasing the percentage of fulvic acids in it; in some soils, it also increases the percentage of humic acids and increases the $C_{HA}:C_{FA}$ ratio and the humin content. But the inhibitor causes a decrease in the percentage of highly condensed humic acids bound to calcium in the humus and increases the abundance but decreases the degree of condensation of the less highly condensed free and sesquioxide-bound humic acids, thus increasing the mobility of the first two humic-acid fractions.

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